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(54) **Freeze-dried composite materials and processes for the production thereof**

Gefriergetrocknete Verbundmaterialien und deren Herstellungsverfahren

Matériaux composites lyophilisés et leur procédé de préparation

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(56) References cited:
WO-A-98/00180 WO-A-98/15299
US-A- 4 320 201

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Description

Field

[0001] The present invention relates to freeze-dried pads comprising a major fraction of a mixture of collagen and oxidized regenerated cellulose (ORC), and to processes for the production of such pads.

Background

[0002] WO98/00180 describes the use of freeze-dried sponges of collagen admixed with oxidized regenerated cellulose (ORC) for the treatment of chronic wounds. Such sponges must in practice meet stringent requirements of purity, sterility and non-antigenicity.

[0003] It has not hitherto been possible to provide sponges of collagen/ORC mixtures having high reproducibility and high tensile strength both when wet and when dry. In particular, collagen is prone to denaturation when it is sterilized by gamma-irradiation. Furthermore, collagen sponges tend to disintegrate rather rapidly in wound fluid, especially in the presence of collagenase enzymes. Whilst this problem can be diminished by chemical cross-linking of the collagen sponge using cross-linking agents such as glutaraldehyde, the use of such cross-linking agents can give rise to problems of toxicity and antigenicity.

Summary of the Invention

[0004] Accordingly, it is an object of the present invention to provide physiologically acceptable, sterile sponge pads based on collagen/ORC mixtures that exhibit high tensile strength.

[0005] It is a further object of the present invention to provide physiologically acceptable, sterile sponge pads based on collagen/ORC mixtures that have very high purity, sterility and low bioburden.

[0006] It is a further object of the present invention to provide physiologically acceptable, sterile sponge pads based on collagen/ORC mixtures that have high uniformity.

[0007] It is a further object of the present invention to provide physiologically acceptable, sterile sponge pads based on collagen/ORC mixtures that exhibit reduced resorption rates under simulated physiological conditions.

[0008] It is a further object of the present invention to provide physiologically acceptable, sterile sponge pads based on collagen/ORC mixtures that exhibit high mechanical strength and long resorption times without chemical cross-linking.

[0009] The present invention provides a sterile freeze-dried sponge, wherein at least 80% by weight of the sponge consists of a mixture of collagen and oxidized regenerated cellulose in the weight ratio 60:40 to 40:60, wherein the sponge has a dry tensile strength of

more than 3N and a wet tensile strength greater than 1 N, and wherein the sponge is obtainable by a process wherein the sponge is sterilized by gamma-irradiation.

[0010] Preferably, the sterility assurance level is better than 10^{-6} .

[0011] The sponge comprises at least 80% by weight of a mixture of collagen and ORC in the weight ratio 60:40 to 40:60. Preferably, the weight ratio contains a small excess of collagen, in a range 50:50 to 40:60 ORC:collagen. Preferably, the freeze-dried sponge consists essentially of collagen, ORC, water and up to 5% of one or more therapeutically active substances such as growth factors. Preferably, the freeze-dried sponge contains no more than 1% by weight of constituents other than collagen, ORC and water.

[0012] The collagen content is determined by hydrolysing the collagen into its constituent amino acids and analyzing for hydroxyproline as detailed below. The collagen content is calculated to be 7.19 times the hydroxyproline content. The ORC content is determined by hydrolysing it to its constituent monosaccharides and analyzing for glucuronic acid as detailed further below.

[0013] Preferably, the freeze-dried sponge has a pH, measured as hereinafter described, of from 2.3 to 4.0, preferably from 2.5 to 3.0.

[0014] Preferably, the sterile freeze-dried sponges according to the present invention have a degree of collagen denaturation, measured as hereinafter described, of less than 15%, preferably less than 10%, and more preferably less than 5%. It is a particularly advantageous feature of the freeze-dried sponges according to the present invention that the collagen is stabilized against denaturation by the gamma-irradiation used in the sterilizing. The degree of denaturation of the collagen is determined by treatment with trypsin to dissolve the denatured collagen (trypsin does not dissolve native collagen), followed by filtration and quantitation of the hydroxyproline in the filtrate, as detailed further below.

[0015] The sterile freeze-dried sponges according to the present invention have a dry tensile strength (maximum load measured as hereinafter described) greater than 3N, preferably greater than 4N. Preferably, the dry tensile load at 20% extension, measured as hereinafter described, is greater than 2.5N, preferably greater than 3.5N. Preferably, the dry extension at break, measured as hereinafter described, is from 15 to 30%, preferably from 20 to 25%.

[0016] The tensile strength characteristics of the sponges according to the present invention are further characterised by wet strength measurements on samples that have been soaked for 15 minutes in PBS prior to testing. The resulting wet strength maximum load is greater than 1 N, preferably greater than 1.25N. The wet load at 20% extension is greater than 0.1N, preferably greater than 0.2N, most preferably 0.2-0.3N. The wet extension at break is preferably 75-100%, more preferably 80-90%.

[0017] Preferably, the sterile freeze-dried sponges

according to the present invention are not chemically cross-linked. They may have some dehydrothermal cross-linking as a result of the freeze-drying process, but preferably there is no chemical cross-linking by glutaldehyde or the like. This reduces the antigenicity and processing costs of the sponges. The present invention achieves satisfactory physical properties of the sponges and sufficiently long resorption times *in vivo* by very careful control of the composition and manufacturing conditions of the sponges. In particular, the sponges preferably contain ORC fibers, wherein a volume fraction of at least 80% of the fibers have lengths in the range of 20 μ m to 1000 μ m. Such a size distribution can be achieved, for example, by milling an ORC cloth, followed sieving the milled powder to remove fibers outside the range. Preferably, the average (mean by volume) length of the ORC fibers is in the range 250 μ m to 450 μ m.

[0018] The selection of ORC fiber lengths in this range results in easy mixing of the ORC and collagen and highly homogeneous products. The ORC is more thoroughly complexed with the collagen, which results in enhanced therapeutic properties of the sponge. Furthermore, the ORC is more effective to reduce denaturation of the collagen by gamma-radiation during sterilization. Surprisingly, these advantages can be achieved while maintaining the tensile strength of the sponge despite the small size of the ORC fibers.

[0019] The desired physicochemical properties of the freeze-dried sponges according to the present invention are further achieved by the use of collagen that has undergone sequential alkali and acid treatment steps to purify the collagen substantially without denaturing the collagen fibers. Preferably, the bioburden (TVC) of the freeze-dried sponge according to the present invention is less than 100cfu/g, more preferably less than 10cfu/g, and most preferably less than 1cfu/g.

[0020] The sterile freeze-dried sponges according to the present invention have high and uniform porosity, and a high liquid absorption capacity. The measured absorption of the uncompressed pads in 0.9% saline is preferably greater than 12g/100cm², more preferably greater than 15g/100cm².

[0021] Preferably, the sterile freeze-dried sponge according to the present invention has a resorption time under simulated physiological conditions as described in more detail below of more than 48 hours.

[0022] The present invention further provides a method of manufacture of a freeze-dried sponge pad comprising the steps of:-

providing an acidified paste of purified collagen fibers, wherein the collagen is less than 10% denatured;
providing oxidized regenerated cellulose fibers, wherein at least 80% of said fibers have lengths in the range of 20 μ m to 1000 μ m;
combining said collagen and said ORC fibers in a

homogeneous aqueous dispersion in a weight ratio of 60:40 to 40:60 collagen:ORC, said aqueous dispersion being acidified to a pH in the range of 2.8 to 3.2 and having a total solids concentration of 0.8 to 1.2% by weight;

pouring said aqueous dispersion into trays to a depth greater than 1cm;

freezing the dispersion to a temperature less than -30°C, followed by a temperature programmed freeze drying and dehydrothermal cross-linking to a final moisture content of 5-15% by weight;

splitting the freeze-dried dispersion to remove surface layers and leave one or more pads; and sterilizing the one or more pads by gamma-irradiation.

[0023] Preferably, the process according to the present invention is carried out substantially without the use of any chemical cross-linking agents.

[0024] Preferably, the step of providing collagen comprises the following steps:-

providing fresh and unswollen splits of bovine corium;

treating the corium splits with a solution containing sodium hydroxide and hydrogen peroxide to swell and sterilize the corium; then

treating the corium with a aqueous alkali solution at a pH greater than 12 and temperature less than 50°C for a period of 10-14 days; then

treating the corium with a aqueous acid solution at a pH of 0.8-1.2 and temperature less than 50°C until the pH of the corium splits drops to less than 2.5; then

washing the corium, and comminuting the corium with sufficient water to form a paste.

[0025] This treatment results in a collagen of exceptional purity and uniformity, without significant denaturing of the collagen. The collagen paste may be stored in the frozen state, but preferably the collagen is not freeze-dried intermediate the above steps and the step of combining the collagen with the ORC.

[0026] Preferably, the step of providing oxidized regenerated cellulose fibers comprising milling an oxidized regenerated cellulose cloth and screening the milled particles to remove particles having size less than 20 μ m or greater than 1000 μ m.

[0027] Preferably, the step of dispersing the collagen and the ORC comprises the steps of:-

adding an acid-swollen collagen/water paste to acidified water;

adding oxidized regenerated cellulose fibers to the acidified water; and

homogenizing the resulting mixture.

Detailed Description

[0028] The dispersion is poured into trays to a depth of at least 10 mm, preferably at least 20mm, and frozen into blocks in the trays before freeze drying. The freezing is preferably carried out by placing the trays containing the slurry onto pre-chilled shelves at -55°C. The trays are then loaded into a freeze-dryer, held at -50°C for two hours, then at -40°C before starting the freeze-drying cycle. This freezing method gives more uniformly distributed ice crystals, and hence more uniform products, than simply blast freezing the slurry in the trays.

[0029] Preferably, the step of freeze drying is carried out with dehydrothermal cross-linking using a temperature program in the range -40°C to +30°C, to give blocks of freeze dried material. The blocks are split to remove surface layers, and to provide one or more pads. The settling of collagen and ORC fibers in trays results in a desired orientation of collagen and ORC fibers in the final pads. Furthermore, splitting the final pads from a larger block ensures that they have high homogeneity and surface uniformity.

[0030] Preferably, the step of sterilizing is carried out by gamma-irradiation at a dose of 18-29 KGy. It has been found that surprisingly little denaturation of the collagen takes place in the sterilizing step, which may be due to a stabilizing effect of the ORC.

[0031] In preferred embodiments of the process according to the present invention, the weight ratio of collagen to oxidized regenerated cellulose is from 50:50 to 55:45 and the pH of the aqueous dispersion is from 2.9 to 3.1.

[0032] A specific embodiment of the process and product according to the present invention will now be described further, by way of example.

Example 1

[0033] A freeze-dried collagen/ORC sponge is prepared as follows.

[0034] First, the collagen component is prepared from bovine corium as follows. Bovine corium is split from cow hide, scraped and soaked in sodium hypochlorite solution (0.03% w/v) to inhibit microbial activity pending further processing.

[0035] The corium is then washed with water and treated with a solution containing sodium hydroxide (0.2% w/v) and hydrogen peroxide (0.02% w/v) to swell and sterilize the corium at ambient temperature.

[0036] The corium splits then undergo an alkali treatment step in a solution containing sodium hydroxide, calcium hydroxide and sodium bicarbonate (0.4% w/v, 0.6% w/v and 0.05% w.v, respectively) at pH greater than 12.2, ambient temperature, and for a time of 10-14 days, with tumbling, until an amide nitrogen level less than 0.24mmol/g is reached.

[0037] The corium splits then undergo an acid treatment step with 1 % hydrochloric acid at ambient tem-

perature and pH 0.8-1.2. The treatment is continued with tumbling until the corium splits have absorbed sufficient acid to reach a pH less than 2.5. The splits are then washed with water until the pH value of corium splits reaches 3.0-3.4.

[0038] The corium splits are then comminuted with ice in a bowl chopper first with a coarse comminution and then with a fine comminution setting. The resulting paste, which is made up in a ratio of 650g of the corium splits to 100g of water, as ice, is frozen and stored before use in the next stage of the process. However, the collagen is not freeze-dried before admixture with the ORC in the next stage.

[0039] The ORC component of the freeze-dried pad is prepared as follows. A SURGICEL® cloth (Johnson & Johnson Medical, Arlington) is milled using a rotary knife cutter through a screen-plate, maintaining the temperature below 60°C.

[0040] The milled ORC powder and the required weight (according to solids content) of frozen collagen paste are then added to a sufficient amount of water acidified with acetic acid to obtain a pH value of 3.0 and a total solids content of 1.0%. The mixture is homogenized through a Fryma® MZ130D homogenizer, progressively diminishing the settings to form a homogeneous slurry. The pH of the slurry is maintained at 2.9-3.1. The slurry temperature is maintained below 20°C, and the solids content is maintained at 1% ± 0.07.

[0041] The resulting slurry is pumped to a degassing vessel. Vacuum is initiated for a minimum of 30 minutes, with intermittent stirring, to degas the slurry. The slurry is then pumped into freeze-drier trays to a depth of 25mm. The trays are placed onto freezer shelves where the temperature has been preset to -40°C. The freeze-drier programme is then initiated to dry and dehydrothermally cross-link the collagen and ORC to form thick sponge pads.

[0042] On completion of the cycle, the vacuum is released, the freeze-dried blocks are removed, and are then split to remove the top and bottom surface layers, and to divide the remainder of the blocks into 3mm-thick pads. The step of splitting the 5 freeze-dried blocks into pads is carried out with a Fecken Kirfel K1 splitter.

[0043] Finally, the pads are die-cut to the desired size and shape on a die-cutter, packaged, and sterilized with 18-29 KGy of cobalt 60 gamma-irradiation. Surprisingly, this irradiation does not cause significant denaturation of the collagen, which appears to be stabilized by the presence of ORC.

[0044] The resulting freeze-dried collagen ORC pads have a uniform, white, velvety appearance. The thickness of the pads is 3.2 ± 0.17 mm (N = 8 batches). The collagen content is $54\% \pm 3.8\%$ (N = 12 batches). The hydroxyproline content is $7.6 \pm 0.5\%$ (N = 12 batches). The carboxylate content is $10.98 \pm 0.81\%$ (N = 12 batches). The ash content is $0.16 \pm 0.1\%$ (N = 12 batches). The heavy metals (lead) content is less than 1 ppm. The pH is 2.78 ± 0.15 . The denaturation level is $4.87 \pm$

1.54%. The endotoxin level is 33.5 ± 0.9 cfu/g. The bioburden level is 0.2 ± 0.3 cfu/g. The moisture content (loss on drying) is $12.0 \pm 12.8\%$.

Procedure 1

[0045] The collagen content of the materials according to the present invention is measured as follows:-

[0046] Briefly, collagen is hydrolysed into constituent amino acids. The amount of the amino acid hydroxyproline is determined by oxidizing with chloramine-T and then coupling with 4-dimethylamino-benzaldehyde to produce a coloured product, the concentration of which is measured spectrophotometrically at 550 nanometers.

[0047] Hydrolysis of the samples is carried out with 6 molar hydrochloric acid at 105°C until digestion is completed, which takes at least 16 hours. The solution is then neutralized to pH 6 with 6 molar NaOH solution. The solution is then diluted.

[0048] Typically, for a 10 mg sample, the procedure uses 1 ml of 6 molar HCl, and the final volume for analysis on dilution is 500 ml.

[0049] A 1.0 ml sample of the test solution is treated with 1.0 ml of an oxidant solution 5 prepared by dissolving 7 gm of chloramine-T in 600 ml of citrate buffer. The mixture is allowed to stand for 10 minutes, after which 1.0 ml of 20% perchloric acid is added, mixed and allowed to stand for 5 minutes at room temperature.

[0050] The mixture is then treated with 1.0 ml of a colour reagent prepared by dissolving 30 gm of 4-dimethylamino benzaldehyde in 45 ml of perchloric acid (60% w/v) followed by dilution in 250 ml of propane-2-ol. The mixture was treated in a water bath at 60°C for 20 minutes, cooled for 5 minutes, followed by reading the optical density at 550 nanometers. The optical density is compared against values measured for control samples of pure collagen at various concentrations, pure hydroxyproline at various concentrations, and blank control samples to arrive at the hydroxyproline content.

[0051] The collagen content of the sample in weight percent is obtained by multiplying the measured hydroxyproline content in weight percent by 7.19.

Procedure 2

[0052] The amount of denatured collagen present in the materials according to the present invention is determined as follows.

[0053] Briefly, native collagen is protected by its triple-helical structure against proteolytic enzymes except for specific collagenases. If the helical structure is damaged, the resulting denatured collagen is susceptible to other proteases, such as trypsin, and is degraded to peptides. In this procedure, trypsin-resistant native collagen is separated from the degraded peptides by salt precipitation, and non-native collagen present in the filtrate is quantitated by hydroxyproline analysis.

[0054] A sample of the material according to the in-

vention (100 mg) is weighed into a 50 ml conical flask. To the flask is added 10 ml of tris-HCl buffer solution containing 500 units trypsin. Blank experiments without the trypsin enzyme are also run. The mixtures are shaken at 4°C for 5 hours. Then 2.5 ml of 25% NaCl in 3 molar acetic acid are added to each container and mixed thoroughly. The containers are then placed in a refrigerator at 4°C for a minimum of 16 hours. The chilled extract is filtered through Whatman 541 filter paper into 50 ml beaker, and the hydroxyproline content of the sample of the filtrate is measured by the method according to Procedure 1. Denatured collagen is calculated as 7.19 x the measured hydroxyproline level, and the percentage of denatured collagen is calculated by comparison with the total collagen content measured by Procedure 1.

Procedure 3

[0055] The ORC content of materials according to the present invention is measured by a method similar to that described by Bitter and Muir in *Analytical Chemistry* vol. 4 (1962), pages 300-334.

[0056] Briefly, the material is hydrolysed to its individual constituents using sulphuric acid. Upon hydrolysis, the ORC breaks down to glucuronic acid (approximately 80%) and glucose (20%). The glucuronic acid residues then undergo a colour reaction with carbozole, the absorbance of which is measured against a series of ORC standards to give an estimation of the ORC content.

[0057] Samples of the material under test (10 mg) are placed in hydrolysate tubes. Deionised water (0.5 ml) and concentrated sulphuric acid (3 ml) are added, and the mixture is mixed on a vortex mixer for 15 minutes and checked for complete dissolution of the sample.

[0058] An aliquot (0.1 ml) of each sample hydrolysate is added to 2.9 ml of sodium tetraborate solution (0.025 molar in concentrated sulphuric acid) and mixed using vortex mixer. The sample tubes are placed in a boiling water bath for 10 minutes, and then cooled. Then 0.1 ml of carbozole solution (0.125% in ethanol) is added to each tube and mixed thoroughly with a vortex mixer, followed by placing the tubes in a boiling water bath for 15 minutes and cooling. The absorbance of the resulting solutions at 523 nanometers is then measured against a zero concentration ORC standard.

Procedure 4

[0059] The number of bacteria, fungi or yeast organisms present in the materials according to the present invention is measured as follows.

[0060] A 2 gm sample of the material is extracted with 100 ml of sterile one-quarter strength Ringer's solution, and an aliquot (5 ml) is passed to a sterile membrane filter (pore size 0.45 m) for sterile filtration. The filters are placed onto a nutrient medium in a Petri dish, and incubated under sterile conditions for 48 hours at 30°C

to allow the growth of germ colonies which can be counted with the naked eye or under a stereomicroscope if necessary. Appropriate control blanks are also run.

[0061] The level of microbiological contamination of the samples is expressed as the total viable count (TVC) in colony forming units per gram (cfu/g) in accordance with the following formula:

$$\text{TVC} = [(N \times 100)/(5 \times W)]$$

where N is the count of colonies, W is the weight of the sample in grams, 100 is the volume of the extractant solution in ml, and 5 is the volume of the aliquot (5ml) that is filtered.

Procedure 5

[0062] The wet and dry tensile strengths of the material according to the invention are measured as follows.

[0063] Samples are die cut from a 3mm thickness pad of the material. The sample dimensions are 2.5 x 12 cm. The samples are loaded into an Instron tensile tester with jaw face dimensions 50 x 25 mm. The dry tensile strength is measured as the load at 20% elongation and the load at break. The extension at break is expressed as the percentage of the initial jaw separation: A minimum of 5 specimens was tested.

The wet tensile measurements are carried out in the same way on samples that have been soaked for 15 minutes in phosphate buffered saline (PBS).

Procedure 6

[0064] The pH of solid materials according to the present invention is measured by macerating 100 gm of the material in 100 ml of deionised water, and measuring the pH of the resulting slurry with a glass electrode.

Procedure 7

[0065] The resorption rate of the composite materials according to the invention is measured in a flow of simulated wound fluid as follows.

[0066] A circular pad of the material under test, of thickness 3mm and diameter 6cm is placed in a cylindrical recess and covered with a layer of liquid-impermeable backing material. Simulated wound fluid (3.45 mg/l collagenase in phosphate buffered saline) was pumped radially at a rate of 2.5 ml, 7.5 ml or 12 ml/24 hours from an opening below the centre of the disk under test to six openings disposed radially around the edges of the disk under test, below the disk under test, to simulate low, medium and high wound exudate flow rates. The time to resorption was estimated as the time required for complete dissolution of the pad under test. This time was at least two days for the high flow rate, at least three days for the medium flow rate, and at least

six days for the low flow rate.

Procedure 8

[0067] The liquid absorbency of the materials according to the present invention was measured as follows.

[0068] A sample (typically 2.5 cm x 2.5 cm x 0.3 cm) of the material under test was weighed dry, and then immersed in phosphate buffered saline (PBS) for 15 minutes, removed with tweezers, and weighed again. The liquid absorbency was then calculated in grams of absorbed liquid per gram (dry weight) of the material.

Procedure 9

[0069] The levels of bacterial endotoxin in the materials according to the present invention are determined as follows.

[0070] Briefly, endotoxins from gram-negative bacterial cell walls cause limulus amoebocyte lysate (LAL) to gel. The test is conducted as a limit test, wherein the sample is determined to be positive or negative to the test, judged against preestablished endotoxin concentrations. Positive and negative controls are essential and are carried out with each test run. The control standard endotoxin has its potency verified against referenced standard endotoxin (USP EC6). Details of the method can be found in Carl Freudentberg Method 091,102 (pyrogenicity); in USP XXIII (1985); in the FDA Guidelines 1987 and in European Pharmacopoeia 2.6.14 (1998).

Claims

1. A sterile freeze-dried sponge, wherein at least 80% by weight of the sponge consists of a mixture of collagen and oxidized regenerated cellulose in the weight ratio 60:40 to 40:60, wherein the sponge has a dry tensile strength of more than 3N and a wet tensile strength greater than 1 N, and wherein the sponge is obtainable by a process wherein the sponge is sterilized by gamma-irradiation.
2. A sterile freeze-dried sponge according to claim 1, wherein the sponge is substantially free of chemical cross-links.
3. A sterile freeze-dried sponge according to claim 1, wherein the collagen has a degree of denaturation less than 20%.
4. A sterile freeze-dried sponge according to claim 3, wherein the collagen has a degree of denaturation less than 10%.
5. A sterile freeze-dried sponge according to claim 3, wherein 80% of the oxidized regenerated cellulose

fibers by volume are between 20 μ m and 1000 μ m in length.

6. A sterile freeze-dried sponge according to claim 5, wherein the mean length of the oxidized regenerated cellulose fibers is from 250 to 450 μ m.

7. A sterile freeze-dried sponge according to claim 1, wherein the oxidized regenerated cellulose is in the form of fibers, and at least 90% of the fibers by volume are less than 1mm in length.

8. A sterile freeze-dried sponge according to claim 1, wherein the ratio of collagen to oxidized regenerated cellulose is from 50:50 to 55:45.

9. A sterile freeze-dried sponge according to claim 1, wherein the bioburden (TVC) of the sponge is less than 100 cfu/g, preferably less than 10 cfu/g, more preferably less than 1 cfu/g.

10. A sterile freeze-dried sponge according to claim 1, wherein the sponge contains from 5 to 15% by weight of water.

11. A sterile freeze-dried sponge according to claim 1, wherein a layer of the sponge of thickness 3mm has an uncompressed absorption capacity of 0.9% saline of from 15 to 20g/100cm².

12. A sterile freeze-dried sponge according to claim 1, wherein the sponge has a resorption time under simulated physiological conditions of more than 48 hours.

13. A method of manufacture of a freeze-dried sponge pad comprising the steps of:-

providing an acidified paste of purified collagen fibers, wherein the collagen is less than 10% denatured;
providing oxidized regenerated cellulose fibers, wherein at least 80% of said fibers have lengths in the range of 20 μ m to 1000 μ m;
combining said collagen and said ORC fibers in a homogeneous aqueous dispersion in a weight ratio of 60:40 to 40:60 collagen:ORC, said aqueous dispersion being acidified to a pH in the range of 2.8 to 3.2 and having a total solids concentration of 0.8 to 1.2% by weight;
pouring said aqueous dispersion into trays to a depth greater than 1cm;
freezing the dispersion to a temperature below -30°C, followed by a temperature programmed freeze drying and dehydrothermal cross-linking to a final moisture content of 5-15% by weight;
splitting the freeze-dried dispersion to remove surface layers and leave one or more pads; and

sterilizing the one or more pads by gamma-irradiation.

14. A method according to claim 13 carried out substantially without the use of any chemical cross-linking agents.

15. A method according to claim 13, wherein the step of providing collagen comprises the following steps:-

providing fresh and unswollen splits of bovine corium;
treating the splits with a hypochloride solution to inhibit microbial activity;
treating the corium with a solution containing sodium hydroxide and hydrogen peroxide to swell and sterilize the corium; then
treating the corium with an aqueous alkali solution at a pH greater than 12 and temperature less than 50°C for a period of ten-fourteen days; then
treating the corium with an aqueous acid solution at a pH of 0.8-1.2 and temperature less than 50°C; then
washing the corium, and comminuting the corium with sufficient water to form a paste.

16. A method according to claim 13, wherein the step of providing oxidized regenerated cellulose fibers comprising milling an oxidized regenerated cellulose cloth and screening the milled particles to remove particles having size less than 20 μ m or greater than 1000 μ m.

17. A method according to claim 13, wherein the step of dispersing the collagen and the ORC comprises the steps of:-

adding an acid-swollen collagen/water paste to acidified water;
adding oxidized regenerated cellulose fibers to the acidified water; and
homogenizing the resulting mixture.

18. A method according to claim 13, wherein the step of freezing is carried out by placing the trays containing the aqueous dispersion onto chilled shelves in a freezer followed by holding the trays at a temperature below -30°C until the freezing is complete.

19. A method according to claim 13, wherein the step of freeze drying is carried out with dehydrothermal cross-linking.

20. A method according to claim 13, wherein the step of sterilizing is carried out by gamma-irradiation at a dose of 18-29 KGy.

21. A method according to claim 13, wherein the weight ratio of collagen to oxidized regenerated cellulose is from 50:50 to 55:45 and the pH of the aqueous dispersion is from 2.9 to 3.1.

Patentansprüche

1. Steriler gefriergetrockneter Schwamm, wobei mindestens 80Gew.% des Schwammes aus einem Gemisch von Kollagen und oxidierte regenerierte Zellulose in einem Gewichtsverhältnis von 60:40 bis 40:60 besteht, wobei der Schwamm eine trockene Zugfestigkeit von mehr als 3N aufweist und eine nasse Zugfestigkeit von mehr als 1N aufweist und wobei der Schwamm durch ein Verfahren erhältlich ist, bei dem der Schwamm durch Gamma-Bestrahlung sterilisiert wird. 10
2. Steriler gefriergetrockneter Schwamm nach Anspruch 1, wobei der Schwamm im wesentlichen frei von chemischen Kreuzvernetzungen ist. 20
3. Steriler gefriergetrockneter Schwamm nach Anspruch 1, wobei das Kollagen ein Ausmaß an Denaturierung von weniger als 20% aufweist. 25
4. Steriler gefriergetrockneter Schwamm nach Anspruch 3, wobei das Kollagen ein Ausmaß an Denaturierung von weniger als 10% aufweist. 30
5. Steriler gefriergetrockneter Schwamm nach Anspruch 3, worin 80% der oxidierten regenerierten Zellulosefasern pro Volumen zwischen 20 µm und 1000 µm lang sind. 35
6. Steriler gefriergetrockneter Schwamm nach Anspruch 5, wobei die mittlere Länge der oxidierten regenerierten Zellulosefasern von 250 bis 450 µm ist. 40
7. Steriler gefriergetrockneter Schwamm nach Anspruch 1, wobei die oxidierte regenerierte Zellulose in der Form von Fasern vorliegt und mindestens 90% der Fasern pro Volumen weniger als 1 mm lang sind. 45
8. Steriler gefriergetrockneter Schwamm nach Anspruch 1, wobei das Verhältnis von Kollagen zu oxidierte regenerierte Zellulose von 50:50 bis 55:45 ist. 50
9. Steriler gefriergetrockneter Schwamm nach Anspruch 1, wobei die Biobelastung (TVC) des Schwammes weniger als 100 cfu/g, bevorzugterweise weniger als 10 cfu/g, weiter bevorzugt weniger als 1 cfu/g ist. 55

10. Steriler gefriergetrockneter Schwamm nach Anspruch 1, wobei der Schwamm von 5 bis 15Gew.% Wasser enthält.

- 5 11. Steriler gefriergetrockneter Schwamm nach Anspruch 1, wobei eine Lage des Schwammes mit einer Dicke von 3 mm eine nicht komprimierte Absorptionskapazität von 0,9% Kochsalzlösung von 15 bis 20 g/100 cm² aufweist.

- 10 12. Steriler gefriergetrockneter Schwamm nach Anspruch 1, wobei der Schwamm eine Resorptionszeit unter simulierten physiologischen Bedingungen von mehr als 48 Stunden aufweist.

- 15 13. Verfahren zur Herstellung eines gefriergetrockneten Schwammkissens, umfassend die Schritte von:

zur Verfügung stellen einer angesäuerten Paste aus gereinigten Kollagenfasern, wobei das Kollagen weniger als 10% denaturiert ist;

zur Verfügung stellen von oxidierten regenerierten Zellulosefasern, wobei mindestens 80% der Fasern Längen im Bereich von 20 µm bis 1000 µm aufweisen;

Kombinieren des Kollagens und der ORC-Fasern in einer homogenen wässrigen Dispersion in einem Gewichtsverhältnis von 60:40 bis 40:60 Kollagen:ORC, wobei die wässrige Dispersion auf einen pH im Bereich von 2,8 bis 3,8 angesäuert wird und eine gesamte Feststoffkonzentration von 0,8 bis 1,2Gew.% aufweist;

Gießen der wässrigen Dispersion in Tablett auf eine Tiefe von mehr als 1 cm;

Frieren der Dispersion auf eine Temperatur unterhalb von 30°C, gefolgt von einem Temperatur-programmierten Gefriertrocknen und dehydothermalen Kreuzvernetzen auf einen finalen Feuchtigkeitsgehalt von 5-15Gew.%;

Aufteilen der gefriergetrockneten Dispersion, um Oberflächenlagen zu entfernen und ein oder mehrere Kissen zurückzulassen; und

Sterilisieren der einen oder mehreren Kissen durch Gamma-Bestrahlung.

14. Verfahren nach Anspruch 13, das im wesentlichen ohne Verwendung von chemischen kreuzvernetzenden Mitteln durchgeführt wird.

15. Verfahren nach Anspruch 13, wobei der Schritt von zur Verfügung stellen von Kollagen die folgenden Schritte umfaßt:

zur Verfügung stellen von frischen und nicht gequollenen Stücken von Rinderkorium;

Behandeln der Stücke mit einer Hypochloridlösung, um eine mikrobielle Aktivität zu inhibieren;

Behandeln des Koriums mit einer Lösung, die Natriumhydroxid und Wasserstoffperoxid enthält, um das Korium aufzuquellen und zu sterilisieren; dann

Behandeln des Koriums mit einer wässrigen Alkalilösung bei einem pH von größer als 12 und einer Temperatur von niedriger als 50°C für eine Zeitdauer von 10-14 Tagen; dann

Behandeln des Koriums mit einer wässrigen sauren Lösung bei einem pH von 0,8-1,2 und einer Temperatur von niedriger als 50°C; dann

Waschen des Koriums und Zermahlen des Koriums mit ausreichend Wasser, um eine Paste zu bilden.

16. Verfahren nach Anspruch 13, wobei der Schritt von zur Verfügung stellen von oxidiertem regeneriertem Zellulosefasern das Mahlen eines oxidierten regenerierten Zellulosegewebes und ein Sieben der gemahlten Partikel umfaßt, um Partikel zu entfernen, die eine Größe von weniger als 20 µm oder größer als 1000 µm aufweisen.

17. Verfahren nach Anspruch 13, wobei der Schritt von Dispergieren des Kollagens und des ORC die Schritte umfaßt von:

Hinzufügen einer Säure-gequollenen Kollagen/Wasser-Paste zu angesäuertem Wasser;

Hinzufügen von oxidierten regenerierten Zellulosefasern zu dem angesäuerten Wasser; und

Homogenisieren des sich ergebenden Gemisches.

18. Verfahren nach Anspruch 13, wobei der Schritt von Gefrieren durch Plazieren der Tablett, die die wässrige Dispersion enthalten, auf gekühlte Regale in einem Gefriergerät durchgeführt wird, gefolgt von Halten der Tablett bei einer Temperatur von unterhalb von -30°C bis zur Vervollständigung des Einfrierens.

19. Verfahren nach Anspruch 13, wobei der Schritt von Gefriertrocknen mit dehydrothermalem Kreuzvernetzen durchgeführt wird.

20. Verfahren nach Anspruch 13, wobei der Schritt von Sterilisieren durch Gamma-Bestrahlung bei einer Dosis von 18-29 KGy durchgeführt wird.

21. Verfahren nach Anspruch 13, wobei das Gewichtsverhältnis von Kollagen zu oxidiertem regeneriertem Zellulose von 50:50 bis 55:45 ist und der pH der wässrigen Dispersion von 2,9 bis 3,1 ist.

Revendications

1. Eponge stérile lyophilisée, dans laquelle au moins 80 % en poids de l'éponge est constitué d'un mélange de collagène et de cellulose régénérée oxydée, dans un rapport en poids de 60 : 40 à 40 : 60, dans laquelle l'éponge a une résistance à la traction à l'état sec qui est supérieure à 3 N et une résistance à la traction à l'état mouillé qui est supérieure à 1 N, et dans laquelle l'éponge peut être obtenue par un procédé dans lequel l'éponge est stérilisée par rayonnements gamma.

2. Eponge stérile lyophilisée selon la revendication 1, dans laquelle l'éponge ne contient substantiellement pas de réticulations chimiques.

3. Eponge stérile lyophilisée selon la revendication 1, dans laquelle le collagène a un degré de dénaturation inférieur à 20 %

4. Eponge stérile lyophilisée selon la revendication 3, dans laquelle le collagène a un degré de dénaturation inférieur à 10 %.

5. Eponge stérile lyophilisée selon la revendication 3, dans laquelle 80 % en volume des fibres de cellulose régénérée oxydée ont une longueur comprise entre 20 µm et 1000 µm.

6. Eponge stérile lyophilisée selon la revendication 5, dans laquelle la longueur moyenne des fibres de cellulose régénérée oxydée va de 250 à 450 µm.

7. Eponge stérile lyophilisée selon la revendication 1, dans laquelle la cellulose régénérée oxydée se présente sous la forme de fibres, et au moins 90 % en volume des fibres ont une longueur inférieure à 1 mm.

8. Eponge stérile lyophilisée selon la revendication 1, dans laquelle le rapport entre le collagène et la cellulose régénérée oxydée va de 50 : 50 à 55 : 45.

9. Eponge stérile lyophilisée selon la revendication 1, dans laquelle la charge biologique (Total Viable Count - TVC) de l'éponge est inférieure à 100 ufc/g, de préférence inférieure à 10 ufc/g, plus préfé-

- rentiellement inférieure à 1 ufc/g.
10. Eponge stérile lyophilisée selon la revendication 1, dans laquelle l'éponge contient de 5 à 15 % en poids d'eau. 5
11. Eponge stérile lyophilisée selon la revendication 1, dans laquelle une couche de l'éponge d'une épaisseur de 3 mm a une capacité d'absorption à l'état non comprimé de 15 à 20 g d'une solution saline à 0,9 % pour 100 cm². 10
12. Eponge stérile lyophilisée selon la revendication 1, dans laquelle l'éponge a un temps de résorption supérieur à 48 heures dans des conditions physiologiques simulées. 15
13. Procédé de fabrication d'un tampon d'éponge stérile lyophilisée comprenant les étapes consistant à : 20
- fournir une pâte acidifiée de fibres de collagène purifié, dans laquelle le collagène est dénaturé à moins de 10 % ;
 - fournir des fibres de cellulose régénérée oxydée, dans lesquelles au moins 80 % desdites fibres ont des longueurs comprises dans la gamme allant de 20 µm à 1000 µm ; 25
 - combiner lesdites fibres de collagène et lesdites fibres de cellulose régénérée oxydée pour donner une dispersion aqueuse homogène avec un rapport en poids entre le collagène et la cellulose régénérée oxydée allant de 60 : 40 à 40 : 60, ladite dispersion aqueuse étant acidifiée à un pH compris dans la gamme allant de 2,8 à 3,2 et ayant une concentration totale en solides de 0,8 à 1,2 % en poids ; 30
 - verser ladite dispersion aqueuse dans des plateaux d'une profondeur supérieure à 1 cm ; 35
 - congeler la dispersion à une température inférieure à - 30°C, et faire suivre par une lyophilisation à température contrôlée et à une réticulation déhydrothermique afin d'atteindre une teneur en humidité finale de 5 à 15 % en poids ; 40
 - fendre la dispersion lyophilisée afin d'éliminer les couches de surface et laisser un ou plusieurs tampons ; et 45
 - stériliser le tampon ou les tampons par rayonnements gamma. 50
14. Procédé selon la revendication 13, effectué en n'utilisant pratiquement pas d'agents chimiques de réticulation. 55
15. Procédé selon la revendication 13, dans lequel l'étape consistant à fournir le collagène comprend les étapes consistant à : 5
- fournir des dédoublements frais et non gonflés de derme bovin ;
 - traiter les dédoublements avec une solution d'hypochlorure afin d'inhiber l'activité microbienne ;
 - traiter le derme avec une solution contenant de l'hydroxyde de sodium et du peroxyde d'hydrogène afin de faire gonfler le derme et de le stériliser ; puis
 - traiter le derme avec une solution aqueuse d'alcali à un pH supérieur à 12 et à une température inférieure à 50°C pendant une période de 10 à 14 jours ; puis
 - traiter le derme avec une solution aqueuse acide à un pH de 0,8 - 1,2 et à une température inférieure à 50°C ; puis
 - laver le derme et broyer le derme avec une quantité d'eau suffisante à l'obtention d'une pâte. 10
16. Procédé selon la revendication 13, dans lequel l'étape consistant à fournir les fibres de cellulose régénérée oxydée comprend le fait de broyer une étoffe de cellulose régénérée oxydée et de tamiser les particules broyées afin d'éliminer les particules ayant une taille inférieure à 20 µm ou supérieure à 1000 µm. 15
17. Procédé selon la revendication 13, dans lequel l'étape consistant à disperser le collagène et la cellulose régénérée oxydée comprend les étapes consistant à : 20
- ajouter une pâte de collagène gonflé à l'acide et d'eau dans de l'eau acidifiée ;
 - ajouter les fibres de cellulose régénérée oxydée dans l'eau acidifiée ; et
 - homogénéiser le mélange qui en résulte. 25
18. Procédé selon la revendication 13, dans lequel l'étape consistant à effectuer une congélation est effectuée en plaçant les plateaux contenant la dispersion aqueuse sur des étagères glacées dans un congélateur, puis en maintenant les plateaux à une température inférieure à - 30°C jusqu'à ce que la congélation soit terminée. 30

19. Procédé selon la revendication 13, dans lequel l'étape de lyophilisation est effectuée avec réticulation déhydrothermique.
20. Procédé selon la revendication 13, dans lequel l'étape de stérilisation est effectuée par rayonnements gamma à une dose de 18 - 29 KGy. 5
21. Procédé selon la revendication 13, dans lequel le rapport en poids entre le collagène et la cellulose régénérée oxydée est de 50 : 50 à 55 : 45 et le pH de la dispersion aqueuse est de 2,9 à 3,1. 10

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